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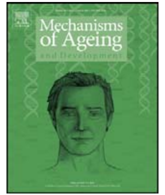

Acute exercise reverses aged-induced impairments in insulin signaling in rodent skeletal muscle

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ABSTRACT

The insulin resistance associated with aging is improved by exercise, but the molecular mechanisms of this improvement are not fully understood. We investigated whether the improvement in insulin action, associated with acute exercise in old rats is dependent on the modulation of pIRS-1Ser307, JNK, IkBα and PTP-1B. Aging rats were subjected to swimming for two 1.5-h long bouts, separated by a 45 min rest period. Sixteen hours after the exercise, the rats were killed and proteins from the insulin signaling pathway were analyzed by immunoblotting. Our results show that the reduction in glucose disappearance rate (Kitt), observed in aged rats, was restored at 16 h after exercise. Aging led to an increase in Ser307 phosphorylation of IRS-1, and this was reversed by exercise in the skeletal muscle, in parallel with a reduction in pJNK and IkBα degradation. Moreover, aging induced an increase in the expression of PTP-1B and attenuated insulin signaling in the muscle of rats, a phenomenon that was reversed by exercise. Interestingly, the decrease in PTP-1B expression in the muscle of exercised old rats was accompanied by an increase in SIRT1 expression. These results provide new insights into the mechanisms by which exercise restores insulin sensitivity during aging.

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1. Introduction

Physical activity and obesity, associated with ageing, appears to play a large role in the regulation of insulin sensitivity with ageing. Many of the effects of ageing on insulin sensitivity are likely related to increased fat mass and decreased physical activity (Rimbert et al., 2004; Amati et al., 2009). On the order hand, endurance training has emerged as an effective intervention for prolonging life span and delaying the installation of degenerative diseases (Paffenbarger et al., 1986; Holloszy, 1988; Blair et al., 1989; Holloszy and Schechtman, 1991; Blain et al., 2000). The mechanism involved in the positive effects of physical activity remains unknown, although a beneficial effect on the age-related development of insulin resistance has been suggested to play an important role (Holloszy, 1988; Carvalho et al., 1996; Blain et al., 2000).

During the last twenty years, a number of defects in insulin signaling have been reported in aging animals (Barnard et al., 1992; Carvalho et al., 1996; Rocha et al., 2003). A decrease in insulin-induced insulin receptor (IR) and insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation levels has been described in the muscle of old rats (Carvalho et al., 1996; Lima et al., 2002), although these alterations may not directly indicate insulin resistance. Moreover, studies have found a reduction in the association of the IRS-1 with phosphatidylinositol 3-kinase (PI3-K) and a decrease in IRS-1 protein levels in old rats (Carvalho et al., 1996; Arias et al., 2001). Notably, the PI3-K/Akt pathway in the skeletal muscle of old rats is also a critical signal defect of insulin dysfunction with aging (Carvalho et al., 1996; Lima et al., 2002).

Many mechanisms may contribute to the dysregulation of the insulin-signaling pathway, including serine phosphorylation of IRS proteins by protein kinases, such as IKappaKinase-β (IKK-β) and c-Jun N-terminal kinase (JNK) (Hotamisligil et al., 1996; Hirosumi

Abbreviations: Akt, protein kinase B/Akt; IkBα, IkappaBalpha Kinase; IR, insulin receptor; IRS, insulin receptor substrate; JNK, c-jun N-terminal Kinase; PI3-K, phosphatidylinositol 3-kinase; PTP-1B, protein tyrosine phosphatase 1B; SIRT1, silent mating type information regulation 2 homolog S. cerevisiae.

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et al., 2002; Ropelle et al., 2006). Much evidence has shown that, during aging, activation of inflammatory signaling pathways such as IKK-β (Kim et al., 2000; Chung et al., 2001) and JNK (Williamson et al., 2003) may occur, leading to phosphorylation of serine sites on IRS-1 and an attenuated insulin signaling pathway. Serine-phosphorylated forms of IRS-1 fail to associate with and activate PI3-K, resulting in the decreased activation of glucose transport and other downstream events (Bouzakri et al., 2005; Karlsson and Zierath, 2007). In addition, it is possible that, during aging, increases in the expression of protein tyrosine phosphatase 1B (PTP-1B) may impair insulin signaling in the skeletal muscle.

Conversely, it is well established that exercise training can improve the insulin sensitivity of young (Luciano et al., 2002), as well as, aged rats (Kern et al., 1992; Arias et al., 2001). However, the molecular mechanisms involved in the improvement in insulin signaling during aging are not fully understood. Recently, we and others have demonstrated that acute exercise reverses insulin resistance in obese rats in parallel with an improvement in insulin signaling (Ropelle et al., 2006; Thyfault et al., 2007; Pauli et al., 2008). These data show that acute exercise attenuates proinflammatory pathways such as JNK and NF-kB prevent insulin resistance in the context of obesity. However, these effects of exercise have not yet been investigated in insulin resistance during aging.

In the present study, we investigated whether the improvement in insulin sensitivity and insulin signaling, via acute exercise, could be associated with modulation of IRS-1Ser307, JNK, IKK-β and PTP-1B in the skeletal muscle of old rats.

2. Materials and methods

2.1. Animals

Male Wistar rats from the University of Campinas Central Animal Breeding Center were used in the experiments. All experiments were approved by the Ethics Committee of the State University of Campinas (UNICAMP). Eight rats (n = 8) were used per group (young group: control rats of 3 months of age), old sedentary rats (group OS: 27 months of age), and exercised 27-month-old rats (group OE).

2.2. Exercise protocol

Rats were accustomed to swimming for 10 min for 2 days. The animals swam in groups of three in plastic barrels of 45 cm in diameter that were filled to a depth of 60 cm, for two 1.5-h long bouts, separated by a 45-min rest period and the water temperature was maintained at ~34 °C. This exercise protocol was adaptated from a previously published procedure (Flores et al., 2006). After the last bout of exercise, animals were fed ad libitum and food was withdrawn 6 h before the tissue extraction, with free access to water. The rats were anesthetized with intraperitoneal injection of sodium thiopental (40 mg/kg (body weight)$^{-1}$) 16 h after the exercise protocol. Following the experimental procedures, the rats were killed under anesthesia (200 mg/kg thiopental), following the recommendations of the NIH.

2.3. Insulin tolerance test (ITT) and serum insulin quantification

For this procedure the animals used were different from the animals used in the tissue extraction and protein analysis by immunoblotting. The ITT was realized 16 h after the exercise protocol and after 6 h of fasting, with free access to water. Briefly, 1.5 UI/kg of human recombinant insulin (Humulin R) from Eli Lilly (Indianapolis, IN, USA) was injected intraperitoneally in anesthetized rats, the blood samples were collected at 0, 5, 10, 15, 20, 25 and 30 min from the tail for serum glucose determination. The rate constant for plasma glucose disappearance (Kitt) was calculated using the formula 0.693/(t1/2). The plasma glucose t1/2 was calculated from the slope of last square analysis of the plasma glucose concentration during the linear phase of declive (Bonora et al., 1989). The plasma glucose level was determined by a colorimetric method using a glucosemeter (Advantage. Boehringer Mannheim, USA). Plasma was separated by centrifugation (1100 × g) for 15 min at 4 °C and stored at −80 °C until assayed. RIA was employed to measure serum insulin, according to a previous description.

2.4. Protein analysis by immunoblotting

As soon as anesthesia was assured by the loss of pedal and corneal reflexes, the abdominal cavity was opened, the portal vein exposed, and 0.2 ml of normal saline with or without insulin (10^{-6} mol/l) was injected. In preliminary experiments, we determined that this dose of insulin can reach peripheral levels that are 3–4 times higher than the dose that can induce the maximal insulin effect on insulin signaling proteins in muscle. At 90 s after the insulin injection, both portions of gastrocnemius (red and white fibers) were ablated, pooled, minced coarsely and homogenized immediately in extraction buffer (1% Triton-X 100, 100 mM Tris, pH 7.4, containing 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF and 0.1 mg of aprotinin/ml) at 4 °C with a Polytron PTA 20S generator (Brinkmann Instruments model PT 10/35) operated at maximum speed for 30 s. The extracts were centrifuged at 9000 × g and 4 °C in a Beckman 70.1 Ti rotor (Palo Alto, CA) for 40 min to remove insoluble material, and the supernatants of these tissues were used for protein quantification, performed by the Bradford method (Bradford, 1976). Proteins were denatured by boiling in Laemmli sample buffer containing 100 mM DTT, run on SDS-PAGE, transferred to nitrocellulose membranes, which were blocked, probed and developed as described previously. The β subunit of the IR (IRβ) and IRS-1 were immunoprecipitated from rat muscle with or without previous insulin infusion. Antibodies used for immunoblotting were anti-phosphotyrosine (pY), anti-IR, anti-IRS-1, anti-Akt, anti-phospho [Ser^{473}] Akt, anti-beta-Actin, anti-c-jun N-terminal kinase (JNK), anti-phospho-JNK, anti-phospho-c-jun, anti-IkBα (Santa Cruz Biotechnology Inc., CA, USA) anti-p85-PI3-kinase (PI3-K), anti-SIRT1 (Cell Signaling Technology, MA, USA), anti-PTP-1B and antiphosphoserine-IRS-1307 (Upstate Biotechnology, NY, USA). Blots were exposed to pre-flashed Kodak XAR film with Cronex Lightning Plus intensifying screens at 80 °C for 12–48 h. Band intensities were quantitated by optical desitometry (Scion Image software, ScionCorp, Frederick, MD) of the developed autoradiographs.

2.5. Statistical analysis

Where appropriate, the results are expressed as means ± SD. Differences between the control group and the old sedentary rat group and between the old sedentary rats and the group submitted to the exercise protocol were evaluated using one-way analysis of variance (ANOVA). When the ANOVA indicated significance, a Bonferroni post hoc test was performed.

3. Results

3.1. Physiological and metabolic parameters

In Table 1, comparative data regarding control (C), old sedentary rats (OS) and old rats submitted to an acute exercise (OE) protocol are presented. Twenty-seven-month-old rats (OS and OE) had a higher body weight and epididymal fat pad weight compared to control rats (C). No significant variations were found in body weight and epididymal fat in OE rats, after a single session of exercise, compared to OS rats. The fasting plasma glucose concentrations were similar between the groups, however serum insulin was higher in old rats (OS and OE), when compared with control rats ($P < 0.01$).

3.2. A single bout of exercise improves insulin signaling in the muscle of old rats

The reduction in glucose disappearance rate (Kitt), observed in old rats, was restored at 16 h after acute exercise (Fig. 1A) ($P < 0.05$). There was a ~35% decrease in IR protein levels in the muscle of old rats, which was reversed by acute exercise. The increase in insulin-induced IRβ tyrosine phosphorylation was less evident in old rats, and exercise partially reversed this alteration. Since protein levels were modulated by aging and physical activity, we determined the ratio between insulin-induced IR tyrosine phosphorylation and protein levels. The phosphate/protein ratio increased 8.7-fold in control animals compared with a 3.0-fold

Table 1
Animal characteristics.

	Control	OS	OE
Body weight (g)	324.7 ± 22.1	573.5 ± 33.8*	575.1 ± 26.5*
Epididymal fat (g/100 g)	1.3 ± 0.07	2.3 ± 0.1*	2.4 ± 0.1*
Blood glucose (mg/dl)	76.8 ± 5.9	87.2 ± 6.9	85.6 ± 9.6
Serum insulin (ng/ml)	2.2 ± 0.1	5.6 ± 0.8*	5.1 ± 0.2*

Data are means ± SEM. Each group was composed of eight animals.
* $P < 0.01$ vs. control group.

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